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PATENT
(700/202D)

Attorney Docket No. 112800.401

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Keith Henry Stockman Campbell et al.

Serial No.: 09/225,233

Filed: January 4, 1999

Group Art Unit: 1632

Examiner: D. Crouch

For: QUIESCENT CELL POPULATIONS FOR NUCLEAR TRANSFER

Commissioner for Patents
Washington, DC 20231

Sir:

DECLARATION OF DR. DAVID WELLS UNDER 37 C.F.R. § 1.132

I, David Wells, declare that:

1. Since 1992, I have held the position of Research Scientist, AgResearch, Ruakura, New Zealand. I am a principal researcher in the field of nuclear transfer with embryonic and somatic cells and have been involved in the establishment of embryonic stem (ES) cell technology at AgResearch. In part, my research involves cloning livestock animals, particularly sheep and cattle, from cultured cells using nuclear transfer into oocytes. I have conducted many studies using nuclear transfer into oocytes and developing the resultant embryos into fetuses and animals. A copy of my Curriculum Vitae is attached hereto.

2. From 1987 to 1988, I held the position of Research Scientist, MAF Technology, Ruakura, New Zealand. During my employment with MAF Technology I developed an embryo splitting technique, which was integrated into a large scale

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multiple ovulation and embryo transfer program conducted at Hopu Hopu Quarantine Research Station in New Zealand. My research involved cloning sheep by embryo splitting. This involved embryo manipulation and culture and the development of identical twin animals by transfer into hosts.

3. In 1991, I graduated with a Doctor of Philosophy from the University of Edinburgh, UK. I conducted the research for my PhD thesis at the Department of Reproduction and Development, AFRC Institute of Animal Physiology and Genetics Research, Roslin Institute, and the Department of Genetics, at the University of Edinburgh. During my PhD program, I worked in Dr. Ian Wilmut's laboratory and developed competence in embryonic stem (ES) cell isolation. My work involved using isolated ES cells to generate germline chimeras by embryo manipulation and culture and the development of animals.

4. I have published numerous manuscripts in the area of cloning livestock animals from cultured cells using nuclear transfer. Two representative manuscripts are: "Production of cloned lambs from an established embryonic cell line: a comparison between in vivo- and in vitro-matured cytoplasts" published in *Biology of Reproduction* 57: 385-393 (1997) and "Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells" published in *Biology of Reproduction* 60: 996-1005 (1999).

5. I have read an article by McLaughlin et al. in *Reproduction Fertil. Develop.* 2, 619-622 (1990), a copy of which is attached hereto.

6. McLaughlin et al. discusses the production of embryos and lambs by nuclear transfer using sheep embryonic cells as nuclear donors.

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7. Based on McLaughlin et al. and on my experience in cloning mammals, the embryos and sheep of McLaughlin et al. do not contain the same set of chromosomes as a single parental mammal. This is because the embryos and sheep of McLaughlin et al. were generated from sheep embryonic cells. The embryos from which these cells were derived were the product of normal sexual reproduction. Consequently, the embryos and sheep of McLaughlin et al. had two parents, one male and one female. The embryos and sheep of McLaughlin et al. had a chromosomal donation from each of these parents. Therefore, embryos and sheep of McLaughlin et al. do not contain the same set of chromosomes as either of their parents.

8. Based on McLaughlin et al. and on my experience in cloning mammals, the embryos and sheep of McLaughlin et al. could be distinguished from an embryo or mammal that contains the same set of chromosomes as a single parental mammal by various techniques. Differences and identities in chromosomes could be readily determined, for example, using the well-known technique of genetic analysis.

9. I have read an article by Prather et al. in *Biology of Reproduction* 41, 414-418 (1989), a copy of which is attached hereto.

10. Prather et al. discusses the production of embryos and pigs by nuclear transfer using pig embryonic cells as nuclear donors.

11. Based on Prather et al. and on my experience in cloning mammals, the embryos and pigs of Prather et al. do not contain the same set of chromosomes as a single parental mammal. This is because the embryos and pigs of Prather et al. were generated from pig embryonic cells. The embryos from which these cells were derived were the product of normal sexual reproduction. Consequently, the embryos and pigs of Prather et al. had two parents, one male and one female. The embryos and pigs of

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Prather et al. had a chromosomal donation from each of these parents. Therefore, embryos and pigs of Prather et al. do not contain the same set of chromosomes as either of their parents.

12. Based on Prather et al. and on my experience in cloning mammals, the embryos and pigs of Prather et al. could be distinguished from an embryo or mammal that contains the same set of chromosomes as a single parental mammal by various techniques. Differences and identities in chromosomes could be readily determined, for example, using the well-known technique of genetic analysis.

13. I have read an article by Yong et al. in *Theriogenology* 35, 299 (1991), a copy of which is attached hereto.

14. Yong et al. discusses the production of embryos and goats by nuclear transfer using goat embryonic cells as nuclear donors.

15. Based on Yong et al. and on my experience in cloning mammals, the embryos and goats of Yong et al. do not contain the same set of chromosomes as a single parental mammal. This is because the embryos and goats of Yong et al. were generated from goat embryonic cells. The embryos from which these cells were derived were the product of normal sexual reproduction. Consequently, the embryos and goats of Yong et al. had two parents, one male and one female. The embryos and goats of Yong et al. had a chromosomal donation from each of these parents. Therefore, embryos and goats of Yong et al. do not contain the same set of chromosomes as either of their parents.

16. Based on Yong et al. and on my experience in cloning mammals, the embryos and goats of Yong et al. could be distinguished from an embryo or mammal that contains the same set of chromosomes as a single parental mammal by various

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techniques. Differences and identities in chromosomes could be readily determined, for example, using the well-known technique of genetic analysis.

17. I have read an article by Cheong et al. in *Biology Reproduction* 48, 958-963 (1993), a copy of which is attached hereto.

18. Cheong et al. discusses the production of embryos and mice by nuclear transfer using mouse embryonic cells as nuclear donors.

19. Based on Cheong et al. and on my experience in cloning mammals, the embryos and mice of Cheong et al. do not contain the same set of chromosomes as a single parental mammal. This is because the embryos and mice of Cheong et al. were generated from mouse embryonic cells. The embryos from which these cells were derived were the product of normal sexual reproduction. Consequently, the embryos and mice of Cheong et al. had two parents, one male and one female. The embryos and mice of Cheong et al. had a chromosomal donation from each of these parents. Therefore, embryos and mice of Cheong et al. do not contain the same set of chromosomes as either of their parents.

20. Based on Cheong et al. and on my experience in cloning mammals, the embryos and mice of Cheong et al. could be distinguished from an embryo or mammal that contains the same set of chromosomes as a single parental mammal by various techniques. Differences and identities in chromosomes could be readily determined, for example, using the well-known technique of genetic analysis.

21. I have read an article by Yang et al. in *Biology Reproduction* 47, 636-643 (1992), a copy of which is attached hereto.

22. Yang et al. discusses the production of embryos and rabbits by nuclear transfer using rabbit embryonic cells as nuclear donors.

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23. Based on Yang et al. and on my experience in cloning mammals, the embryos and rabbits of Yang et al. do not contain the same set of chromosomes as a single parental mammal. This is because the embryos and rabbits of Yang et al. were generated from rabbit embryonic cells. The embryos from which these cells were derived were the product of normal sexual reproduction. Consequently, the embryos and rabbits of Yang et al. had two parents, one male and one female. The embryos and rabbits of Yang et al. had a chromosomal donation from each of these parents. Therefore, embryos and rabbits of Yang et al. do not contain the same set of chromosomes as either of their parents.

24. Based on Yang et al. and on my experience in cloning mammals, the embryos and rabbits of Yang et al. could be distinguished from an embryo or mammal that contains the same set of chromosomes as a single parental mammal by various techniques. Differences and identities in chromosomes could be readily determined, for example, using the well-known technique of genetic analysis.

25. I have read an article by Sims et al. in *Proc. Natl. Acad. Sci.* 90, 6143-6147 (1993), a copy of which is attached hereto.

26. Sims et al. discusses the production of embryos and bovines by nuclear transfer using bovine embryonic cells as nuclear donors.

27. Based on Sims et al. and on my experience in cloning mammals, the embryos and bovines of Sims et al. do not contain the same set of chromosomes as a single parental mammal. This is because the embryos and bovines of Sims et al. were generated from bovine embryonic cells. The embryos from which these cells were derived were the product of normal sexual reproduction. Consequently, the embryos and bovines of Sims et al. had two parents, one male and one female. The embryos

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and bovines of Sims et al. had a chromosomal donation from each of these parents. Therefore, embryos and bovines of Sims et al. do not contain the same set of chromosomes as either of their parents.

28. Based on Sims et al. and on my experience in cloning mammals, the embryos and bovines of Sims et al. could be distinguished from an embryo or mammal that contains the same set of chromosomes as a single parental mammal by various techniques. Differences and identities in chromosomes could be readily determined, for example, using the well-known technique of genetic analysis.

29. I have read WO 95/17500 published June 29, 1995, of Stice et al., a copy of which is attached hereto.

30. Stice et al. discusses the production of genetically modified embryos and mammals by nuclear transfer using genetically modified embryonic cells as nuclear donors.

31. Based on Stice et al. and on my experience in cloning mammals, the genetically modified embryos and mammals of Stice et al. did not receive their chromosomes from a single parental mammal. This is because the genetically modified embryos and mammals of Stice et al. were generated from genetically modified embryonic cells. The embryos from which these cells were derived were the product of normal sexual reproduction. Consequently, the genetically modified embryos and mammals of Stice et al. had two parents, one male and one female. The genetically modified embryos and mammals of Stice et al. had a chromosomal donation from each of these parents. Therefore, the genetically modified embryos and mammals of Stice et al. did not receive their chromosomes exclusively from one of their parents.

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32. Based on Stice et al. and on my experience in cloning mammals, the genetically modified embryos and mammals of Stice et al. could be distinguished from an embryo or mammal that receives its chromosomes from a single parental mammal by various techniques. Differences and identities in chromosomes could be readily determined, for example, using the well-known technique of genetic analysis.

33. Based on my experience with mammals cloned by nuclear transfer and mammals propagated by sexual reproduction, the source of a mammal's chromosomes can be readily determined using genetic analysis. By using genetic analysis, whether a mammal is cloned asexually by somatic cell nuclear transfer or propagated by sexual reproduction, including a mammal produced by nuclear transfer from an embryonic cell, can be determined by comparing the chromosomal DNA of the mammal to that of its parent(s). Only a mammal cloned by somatic cell nuclear transfer will contain the same set of chromosomes as a single parental mammal.

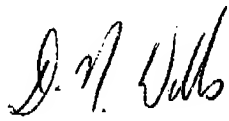
34. Based on my experience with mammals cloned by nuclear transfer and mammals propagated by sexual reproduction, a mammal cloned by somatic cell nuclear transfer is unlike any mammal produced by a process involving sexual reproduction, including a mammal produced by nuclear transfer from an embryonic cell. The set of chromosomes of a mammal cloned by somatic cell nuclear transfer is obtained from a single parental mammal. The set of chromosomes from any mammal produced by a process involving sexual reproduction, including a mammal produced by nuclear transfer from an embryonic cell, comes from two parental mammals, one male and one female. This feature allows the cloned mammal to preserve the genetic information of the parental mammal without dilution.

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35. Based on my experience with cloned mammals, a mammal that contains the same set of chromosomes as a single parental mammal can be distinguished from the parental mammal due to environmental influences. First, the cloned mammal will always be of a younger age than the parental mammal. Second, the cloned mammal will have a variety of phenotypic differences from the parental mammal, for example, differences in fur and skin pigmentation. Third, the cloned mammal will have behavioral differences from the parental mammal.

36. The undersigned further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing therefrom.

Dated: 5th February 2003

By: 
David Wells, Ph.D